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Use of the BACTEC MYCO/F LYTIC Medium for Detection of *Brucella melitensis* Bacteremia

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In a prospective volume-controlled study, the sensitivity of the BACTEC MYCO/F LYTIC medium was similar to that of the Peds Plus/F and Plus Aerobic/F vials for detecting *Brucella melitensis* bacteremia, but the time to detection with the MYCO/F LYTIC medium (101.4 ± 46.7 h) was significantly longer than that with the combined comparator media (65.5 ± 18.9 h, $P = 0.004$).

Studies conducted over the last few years have shown that the BACTEC 9240 blood culture system coupled with the pediatric Peds Plus/F (PPF) or adult Plus Aerobic/F (PAF) medium enables detection of brucellae within the routine 1-week incubation period instituted in most clinical microbiology laboratories and without the need for subculturing negative blood culture vials (2, 4, 7, 9–11).

The BACTEC MYCO/F LYTIC (MFL) medium has been recently developed to improve the recovery of intracellular pathogens such as fungi and mycobacteria (1, 3). Because brucellae are facultative intracellular pathogens, it was speculated that use of the MFL medium might also improve the diagnosis of brucellosis. A prospective study was conducted to assess the performance of the MFL vial for detecting *Brucella melitensis* bacteremia in an area of endemicity and compare it with the PPF and PAF media.

Individuals recently diagnosed with brucellosis by a positive blood or exudate culture and/or a positive *Brucella* antibody test were enrolled in the study after obtaining oral consent from the patient or from his legal guardian. This patient population was chosen to maximize the yield of cultures without the need for including a large number of blood culture sets negative for the organism. If the patient was a child, a 6-ml blood specimen was drawn and divided into two 3-ml aliquots. One aliquot was inoculated into an MFL blood culture bottle, and the second aliquot was inoculated into a PPF bottle. In the case of adults, 10 ml of blood was drawn, of which 5 ml was inoculated into an MFL vial and the remaining volume was inoculated into a PAF vial.

Inoculated vials were incubated in the BACTEC 9240 instrument and monitored for 42 days. Blind terminal subcultures were performed for all negative bottles at the end of the incubation period. Blood culture bottles identified as positive by the instrument were sampled. A Gram stain of the broth was performed, and the fluid was subcultured on sheep blood agar, chocolate agar, and MacConkey plates. Seeded media were incubated at 35°C in a 5% CO_2-enriched atmosphere and examined daily for 4 days. Identification of brucellae was performed as recommended (8).

Blood culture sets were considered positive if *Brucella* organisms were recovered in pure culture from one or both inoculated bottles. The sensitivity rates of MFL and the comparator media were calculated and compared by using the chi-square test. The significance of the differences in the time to detection between vials was assessed by Student's *t* test. In blood culture sets in which both blood culture bottles were positive, time to detection was compared by using the paired *t* test. A *P* value of <0.05 was considered significant in all calculations.

During a 10-month period, a total of 33 blood culture sets were obtained from 32 patients with culture-proven and/or serologically proven brucellosis. Overall, 21 of the 33 (63.6%) blood culture sets yielded *B. melitensis*. No positive blood culture vials were missed by the BACTEC 9240 instrument and detected by terminal blind subcultures. The performance of the MFL and comparator vials is summarized in Table 1. Overall, the MFL vial detected 12 and the PPF detected 14 of 16 positive cultures (sensitivity, 75.0 and 87.5%, respectively; $P > 0.05$). When the MFL and PAF vials were compared, detection rates were 80 and 100%, respectively ($P > 0.05$). In two blood culture sets, only the MFL vial was positive; in five blood culture sets, only the comparator vial was positive; and in 14 sets the organism was detected by both media. The time to detection was 101.4 ± 46.7 h in the 16 positive MFL vials and 65.5 ± 18.9 h in the 19 positive comparator vials ($P = 0.004$). The time to detection in the 14 blood culture sets positive by both media was 99.4 ± 48.3 h for the MFL vials and 60.1 ± 11.9 h for the comparator vials ($P = 0.004$), and in all cases growth was detected earlier in the comparator vial ($P < 0.001$). After 72 h of incubation, 5 of the 14 (35.6%) MFL vials and 13 of the 14 comparator vials (92.9%) were already positive ($P = 0.006$).

Blood culture methods based on lysis of white blood cells have been shown to increase detection of *Brucella* spp. (5, 6, 10–12). Lysis of the blood leukocytes releases already-phagocytosed but still-cultivable organisms, improving recovery of brucellae (11). Lysis-based methods, however, have important drawbacks: they are manual, labor-intensive, and time-consuming and may expose laboratory personnel to highly conta-

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TABLE 1. Isolation and time to detection of brucellae by the MFL and comparator vials

Blood culture set	Time (h) to detection with[a]:		
	MFL vial	Comparator vial	
		PPF	PAF
1	94	65	ND
2	71	54	ND
3	97	57	ND
4	70	58	ND
5	60	54	ND
6	82	52	ND
7	72	59	ND
8	78	60	ND
9	93	72	ND
10	76	28	ND
11	−	129	ND
12	−	61	ND
13	−	72	ND
14	−	68	ND
15	84	−	ND
16	147	−	ND
17	238	ND	75
18	132	ND	69
19	160	ND	72
20	68	ND	67
21	−	ND	73

[a] ND, not done; −, negative result.

gious brucellae (11). It appears, then, that the coupling the MFL medium with the BACTEC 9240 instrument represents the best of two worlds: on the one hand, the saponin contained in the broth causes lysis of the leukocytes into which living brucellae circulate. On the other hand, use of the automated instrument enables continuous monitoring and detection of bacterial growth, minimizes contamination, and improves laboratory safety.

The results of the present study demonstrate, however, that the sensitivity of the MFL vial is similar but not superior to that of PPF and PAF media for the recovery of *B. melitensis*. Although detection of all positive cultures required use of both MFL and traditional vials, this does not necessarily indicate failure of either medium to support growth of some *B. melitensis* strains. It is possible that the increased sensitivity achieved with the combination of two vials was the mere result of drawing a larger blood sample, because brucellar bacteremia is usually of low magnitude, and thus, its detection is highly dependent on the specimen volume (10).

In terms of time to detection, the PPF and PAF vials are significantly faster than the MFL vial, resulting in a clinically important average gain of more than 24 h. The reasons for this difference are not obvious, but it should be noted that in the MFL vial, bacterial growth is detected by measuring CO_2 production, whereas in the comparator bottles, consumption of the oxygen contained in the vial is measured. It is possible that the differences in the vials' performance is the result of monitoring a different end product. The specific composition of the comparator media may also provide better nutritional support to fastidious brucellae, modifying the growth curve and, therefore, shortening the time to detection of the organism.

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